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86879

From: Ford, Vanessa  
Sent: Friday, February 14, 2003 3:28 PM  
To: STIC-Biotech/ChemLib  
Subject: In re: 09677374 Sequence Search

Please run SEQ ID NOs: 1, 3 and 5, please include interference searches.

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Searcher: \_\_\_\_\_  
Phone: \_\_\_\_\_  
Location: \_\_\_\_\_  
Date Picked Up: 2/20  
Date Completed: 2/24  
Searcher Prep/Review: \_\_\_\_\_  
Clerical: \_\_\_\_\_  
Online time: \_\_\_\_\_

TYPE OF SEARCH:  
NA Sequences: 3  
AA Sequences: \_\_\_\_\_  
Structures: \_\_\_\_\_  
Bibliographic: \_\_\_\_\_  
Litigation: \_\_\_\_\_  
Full text: \_\_\_\_\_  
Patent Family: \_\_\_\_\_  
Other: \_\_\_\_\_

VENDOR/COST (where applic.)  
STN: \_\_\_\_\_  
DIALOG: \_\_\_\_\_  
Questel/Orbit: \_\_\_\_\_  
DRLink: \_\_\_\_\_  
Lexis/Nexis: \_\_\_\_\_  
Sequence Sys.: \_\_\_\_\_  
WWW/Internet: \_\_\_\_\_  
Other (specify): \_\_\_\_\_

FILE 'BIOSIS, CABA, CAPLUS, EMBASE, LIFESCI, MEDLINE, SCISEARCH,  
USPATFULL, JAPIO' ENTERED AT 12:13:45 ON 14 FEB 2003

L1 0 S FRYER, JL/AU

FILE 'AGRICOLA, LIFESCI, CONFSCI, BIOSIS, VETU, VETB, PHIN, PHIC' ENTERED  
AT 12:26:13 ON 14 FEB 2003

L2 0 S FRYER, JL/AU

L3 0 S FRYER, ?/SU

L4 2192 S FRYER, ?/AU

L5 26 S L4 AND SALMONIS

L6 19 DUP REM L5 (7 DUPLICATES REMOVED)

L7 0 S L6 AND (17 KDA OR 17 KD OR 17 KILODALTONS)

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(FILE 'HOME' ENTERED AT 11:42:27 ON 14 FEB 2003)

FILE 'AGRICOLA, LIFESCI, CONFSCI, BIOSIS, VETU, VETB, PHIN, PHIC' ENTERED  
AT 11:42:39 ON 14 FEB 2003

L1	0 S KUZYK, MICHAEL/AU
L2	0 S KUZYK, MICHAEL/AU
L3	8 S BURIAN, JAN/AU
L4	0 S KUZYK, MICHAEL/AU
L5	0 S KAY, WILLIAM/AU
L6	1 S THORNTON, JULIAN/AU
L7	91 S KUZYK, ?/AU
L8	64 DUP REM L7 (27 DUPLICATES REMOVED)
L9	6 S L8 AND SALMONIS
L10	109 S PISCIRICKETTSIA SALMONIS
L11	2 S L10 AND (17 KDA)
L12	3 S L10 AND (OUTER SURFACE PROTEIN)

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(FILE 'HOME' ENTERED AT 16:18:14 ON 13 FEB 2003)

FILE 'BIOSIS, CABA, CAPLUS, EMBASE, LIFESCI, MEDLINE, SCISEARCH,  
USPATFULL, JAPIO' ENTERED AT 16:18:26 ON 13 FEB 2003

L1 221 S PISCIRICKETTSIA  
L2 219 S L1 AND SALMONIS  
L3 9 S L2 AND (17 KDA OR 17 KD OR 17 KILODALTONS)  
L4 3 DUP REM L3 (6 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 16:20:29 ON 13 FEB 2003

FILE 'PCTFULL' ENTERED AT 16:21:00 ON 13 FEB 2003

L5 3 S PISCIRICKETTSIA  
L6 3 S L5 AND L2

FILE 'AGRICOLA, LIFESCI, CONFSCI, BIOSIS, VETU, VETB, PHIN, PHIC' ENTERED  
AT 16:22:04 ON 13 FEB 2003

L7 109 S L1 AND L2  
L8 2 S L3

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L9 ANSWER 1 OF 6 AGRICOLA

AB Piscirickettsia salmonis, the etiological agent of salmonid rickettsial septicemia, was purified from infected immortal chinook salmon (Oncorhynchus tshawytscha) embryo cells by a combination of differential and Percoll density gradient centrifugation. Immune sera from rabbits immunized with purified whole cells of P. salmonis reacted with four protein antigens and two carbohydrate antigens with relative molecular sizes of 65, 60, 54, 51, 16, and approximately 11 kDa, respectively. The carbohydrate antigens appear to be mainly core region lipo-oligosaccharide with lesser amounts of lipopolysaccharide. Serum from convalescent rainbow trout (Oncorhynchus mykiss) and coho salmon (Oncorhynchus kisutch) reacted with several minor immunoreactive protein antigens between 10 and 70 kDa in size and a carbohydrate antigen with a relative molecular size of approximately 11 kDa. The salmonid immune system did not appear to elicit a strong humoral response against this intracellular pathogen. Indirect immunofluorescence microscopy, immunogold transmission electron microscopy, and biotin labeling of intact P. salmonis cells suggest that the immunoreactive antigens identified with rabbit antisera are surface exposed and differ significantly from those identified with salmonid antisera.

AN 97:54967 AGRICOLA

DN IND20582168

TI Antigenic characterization of the salmonid pathogen Piscirickettsia salmonis.

AU Kuzyk, M.A.; Thorton, J.C.; Kay, W.W.

CS University of Victoria, BC, Canada.

AV DNAL (QR1.I57)

\*SO Infection and immunity, Dec 1996, Vol. 64, No. 12. p. 5205-5210  
Publisher: Washington, D.C., American Society for Microbiology  
ISSN: 0019-9567

NTE Includes references

CY District of Columbia; United States

DT Article

FS U.S. Imprints not USDA, Experiment or Extension

LA English

L9 ANSWER 2 OF 6 LIFESCI COPYRIGHT 2003 CSA

AB Piscirickettsia salmonis is the aetiological agent of salmonid rickettsial septicemia, an economically devastating rickettsial disease of farmed salmonids. Infected salmonids respond poorly to antibiotic treatment and no effective vaccine is available for the control of P. salmonis. Bacterin preparations of P. salmonis were found to elicit a dose-dependent response in coho salmon (Oncorhynchus kisutch), which varied from inadequate protection to exacerbation of the disease. However, an outer surface lipoprotein of P. salmonis, OspA, recombinantly produced in Escherichia coli elicited a high level of protection in vaccinated coho salmon with a relative percent survival as high as 59% for this single antigen. In an effort to further improve the efficacy of the OspA recombinant vaccine, T cell epitopes (TCE's) from tetanus toxin and measles virus fusion protein, that are universally immunogenic in mammalian immune systems, were incorporated tandemly into an OspA fusion protein. Addition of these TCE's dramatically enhanced the efficacy of the OspA vaccine, reflected by a three-fold increase in vaccine efficacy. These results represent a highly effective monovalent recombinant subunit vaccine for a rickettsia-like pathogen, P. salmonis, and for the first time demonstrate the immunostimulatory effect of mammalian TCE's in the salmonid immune model. These results may also be particularly pertinent to salmonid aquaculture in which the various subspecies are outbred and of heterologous haplotypes.

AN 2001:54329 LIFESCI

TI An efficacious recombinant subunit vaccine against the salmonid rickettsial pathogen Piscirickettsia salmonis

AU Kuzyk, M.A.; Burian, J.; Machander, D.; Dolhaine, D.; Cameron,

S.; Thornton, J.C.; Kay, W.W.; Kurstak E. [editor]  
 CS Canadian Bacterial Diseases Network, Department of Biochemistry and Microbiology, University of Victoria, PO Box 3055, Victoria, British Columbia, V8W 3P6, Canada; E-mail: wkay@uvic.ca  
 SO Vaccine, (20010321) vol. 19, no. 17-19, pp. 2337-2344.  
 Meeting Info.: Millenium Second World Congress on Vaccines and Immunisation. Leige (Belgium). 29 Aug - 3 Sep, 2000.  
 ISSN: 0264-410X.  
 DT Journal  
 TC Conference  
 FS F; V  
 LA English  
 SL English

L9 ANSWER 3 OF 6 LIFESCI COPYRIGHT 2003 CSA  
 AB No effective recombinant vaccines are currently available for any rickettsial diseases. In this regard the first non-ribosomal DNA sequences from the obligate intracellular pathogen *Piscirickettsia salmonis* are presented. Genomic DNA isolated from Percoll density gradient purified *P. salmonis*, was used to construct an expression library in lambda ZAP II. In the absence of preexisting DNA sequence, rabbit polyclonal antiserum raised against *P. salmonis*, with a bias toward *P. salmonis* surface antigens, was used to identify immunoreactive clones. Catabolite repression of the lac promoter was required to obtain a stable clone of a 4,983 bp insert in *Escherichia coli* due to insert toxicity exerted by the accompanying *radA* open reading frame (ORF). DNA sequence analysis of the insert revealed 1 partial and 4 intact predicted ORF's. A 486 bp ORF, *ospA*, encoded a 17 kDa antigenic outer surface protein (*OspA*) with 62% amino acid sequence homology to the genus common 17 kDa outer membrane lipoprotein of *Rickettsia prowazekii*, previously thought confined to members of the genus *Rickettsia*. Palmitate incorporation demonstrated that *OspA* is posttranslationally lipidated in *E. coli*, albeit poorly expressed as a lipoprotein even after replacement of the signal sequence with the signal sequence from *lpp* (Braun lipoprotein) or the rickettsial 17 kDa homologue. To enhance expression, *ospA* was optimized for codon usage in *E. coli* by PCR synthesis. Expression of *ospA* was ultimately improved (similar to 13% of total protein) with a truncated variant lacking a signal sequence. High level expression (similar to 42% tot. prot.) was attained as an N-terminal fusion protein with the fusion product recovered as inclusion bodies in *E. coli* BL21. Expression of *OspA* in *P. salmonis* was confirmed by immunoblot analysis using polyclonal antibodies generated against a synthetic peptide of *OspA* (110-129) and a strong antibody response against *OspA* was detected in convalescent sera from coho salmon (*Oncorhynchus kisutch*).  
 AN 2001:33846 LIFESCI  
 TI *OspA*, a Lipoprotein Antigen of the Obligate Intracellular Bacterial Pathogen *Piscirickettsia salmonis*  
 AU Kuzyk, M.A.; Burian, J.; Thornton, J.C.; Kay, W.W.  
 CS Canadian Bacterial Diseases Network, Department of Biochemistry and Microbiology, University of Victoria, P.O. Box 3055, Victoria, British Columbia, Canada, V8W 3P6; E-mail: wkay@uvic.ca  
 SO Journal of Molecular Microbiology and Biotechnology [J. Mol. Microbiol. Biotechnol.], (20010100) vol. 3, no. 1, pp. 83-93.  
 ISSN: 1464-1801.  
 DT Journal  
 FS J  
 LA English  
 SL English

L9 ANSWER 4 OF 6 CONFSCI COPYRIGHT 2003 CSA  
 AN 1998:11636 CONFSCI  
 DN 98-011636  
 TI Antigenic characterization and vaccinology of *Piscirickettsia salmonis*

\* AU **Kuzyk, M.A.**; Thornton, J.C.; Kay, W.W.  
 CS Dep. Biochemistry and Microbiol., Univ. Victoria, Victoria, British Columbia, Canada V8W 3P6  
 SO European Association of Fish Pathologists, General Secretary, The Marine Laboratory, PO Box 101, Victoria Road, Aberdeen AB11 9DB, Scotland, Abstracts available..  
 Meeting Info.: 973 5010: 8th International Conference of the European Association of Fish Pathologists: Diseases of Fish and Shellfish (9735010). Edinburgh (UK). 14-19 Sep 1997. European Association of Fish Pathologists.  
 DT Conference  
 FS DCCP  
 LA English

L9 ANSWER 5 OF 6 CONFSCI COPYRIGHT 2003 CSA  
 AN 97:50622 CONFSCI  
 DN 97-062601  
 TI Immunodiagnostic tests for Renibacterium salmoninarum and Piscirickettsia salmonis

AU Carlos, S.J.; Thornton, J.C.; Hackett, J.L.; Valdes, F.; Poblete, A.; **Kuzyk, M.A.**; Kay, W.W.  
 SO EAFF General Secretary, Marine Laboratory, PO Box 101 Victoria Road, Aberdeen AB11 9DB, UK, Attn: Dr. David Bruno, Abstracts available. Poster Paper No. P-076.  
 Meeting Info.: 973 5001: 8th International Conference on Diseases of Fish and Shellfish (9735001). Edinburgh (UK). 14-19 Sep 1997. Aquaculture Vaccines, Ltd; Atlantic Veterinary College; BOCM Pauls Ltd.; British Airways; City of Edinburgh; Heriot-Watt University.  
 DT Conference  
 FS DCCP  
 LA English

L9 ANSWER 6 OF 6 CONFSCI COPYRIGHT 2003 CSA  
 AN 97:50468 CONFSCI  
 DN 97-062447  
 TI Antigenic characterization and vaccinology of Piscirickettsia salmonis

\* AU **Kuzyk, M.A.**; Thornton, J.C.; Kay, W.W.  
 SO EAFF General Secretary, Marine Laboratory, PO Box 101 Victoria Road, Aberdeen AB11 9DB, UK, Attn: Dr. David Bruno, Abstracts available. Paper No. O-035.  
 Meeting Info.: 973 5001: 8th International Conference on Diseases of Fish and Shellfish (9735001). Edinburgh (UK). 14-19 Sep 1997. Aquaculture Vaccines, Ltd; Atlantic Veterinary College; BOCM Pauls Ltd.; British Airways; City of Edinburgh; Heriot-Watt University.  
 DT Conference  
 FS DCCP  
 LA English

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